

Amendments to the Claims:

This listing of claims will replace all prior versions and listings of claims in the application.

Listing of Claims:

1. (Withdrawn-Currently Amended) An assay for determining the level of prostacyclin in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of ~~an anti-6-keto-PGF_{1α}~~ primary antibody, a secondary antibody and ~~6-keto-PGF_{1α}-aequorin conjugate~~; an anti-6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF_{1α}-antibody; and a conjugate comprising 6-keto-PGF_{1α} covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys → Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74(Gly74 → Cys) or (Glu76 → Cys), and

wherein the 6-keto-PGF_{1α} is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (2) ~~(3) removing any unbound primary anti-6-keto-PGF_{1α}-antibody and said conjugate 6-keto-PGF_{1α}-aequorin conjugate from the plasma sample following incubation; and~~
- (3) ~~(4) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.~~

2. (Withdrawn-Currently Amended) The assay of claim 1 wherein the ~~secondary anti-immunoglobulin antibody that binds to the anti-6-keto-PGF_{1α}-antibody~~ antibody is coated onto a surface which is exposed to the plasma, ~~primary antibody~~ anti-6-keto-PGF_{1α}-antibody and ~~said 6-keto-PGF_{1α} conjugate.~~

3. (Cancelled).

4. (Withdrawn) The assay of claim 1 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

5. (Withdrawn-Currently Amended) The assay of claim 1 wherein the concentration of said conjugate 6-keto-PGF_{1α}-aequorin-conjugate in the assay is about 1×10^{-10} M.

6. (Cancelled).

7. (Cancelled).

8. (Withdrawn-Currently Amended) A method of determining an appropriate dose of prostaglandin for the treatment of primary pulmonary hypertension in a patient comprising

(1) providing a plasma sample from the patient;

(2) incubating the plasma sample with an effective amount of ~~anti-6-keto-PGF_{1α}-primary antibody, a secondary antibody and 6-keto-PGF_{1α}-aequorin-conjugate;~~

an anti-6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF_{1α}-antibody; and a conjugate comprising 6-keto-PGF_{1α} covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys → Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74(Gly74 → Cys) or (Glu76 → Cys), and

wherein the 6-keto-PGF_{1α} is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine,

(3) removing any unbound ~~primary~~ anti-6-keto-PGF_{1α}-antibody and said conjugate from the plasma sample following incubation;

(4) measuring and correlating the amount of detected 6-keto- PGF_{1α} with the appropriate dosage of prostaglandin for the patient.

9. (Withdrawn-Currently Amended) The method of claim 8 wherein the ~~secondary antibody~~ anti-immunoglobulin antibody is coated onto a surface which is exposed to the plasma, ~~primary anti-6-keto-PGF_{1 α} antibody and 6-keto-PGF_{1 α} -aequorin~~ said conjugate.

10. (Cancelled).

11. (Withdrawn) The assay of claim 8 wherein the plasma sample is obtained from a patient receiving intravenous prostaglandin therapy.

12. (Withdrawn-Currently Amended) The assay of claim 8 wherein the concentration of ~~6-keto-PGF_{1 α} -aequorin~~ said conjugate in the assay is about 1×10^{-10} M.

13. (Withdrawn-Currently Amended) An assay for determining the level of a biomolecule in plasma comprising:

- (1) providing a plasma sample;
- (2) incubating the plasma sample with an effective amount of ~~a primary antibody~~ an anti-6-keto-prostaglandin F_{1 α} (6-keto-PGF_{1 α}) antibody to the biomolecule, ~~a secondary antibody~~ an anti-immunoglobulin antibody that binds to the biomolecule and a biomolecule-aequorin conjugate comprising 6-keto-PGF_{1 α} covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys \rightarrow Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 \rightarrow Cys), 70 (Gly70 \rightarrow Cys), 74 (Gly74 \rightarrow Cys) or (Glu76 \rightarrow Cys), and

wherein the 6-keto-PGF_{1 α} is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (2) ~~(3)~~ removing any unbound ~~primary antibody~~ anti-6-keto-PGF_{1 α} antibody and biomolecule-aequorin conjugate from the plasma sample following incubation; and
- ~~(3)~~ (4) measuring and correlating light intensity of the plasma sample with amount of biomolecule within the plasma sample.

14. (Withdrawn-Currently Amended) The assay of claim 13 wherein the ~~secondary antibody anti-immunoglobulin antibody~~ is coated onto a surface which is exposed to the plasma, ~~primary antibody anti-6-keto-PGF_{1α} antibody~~ and biomolecule-aequorin conjugate.

15-18. (Cancelled).

19. (Withdrawn): The biomolecule aequorin conjugate of claim 17 wherein the biomolecule is a peptide.

20. (Withdrawn-Currently Amended) A method for determining the effect of a therapeutic agent on the level of prostacyclin in the plasma of a patient comprising

- (1) administering the therapeutic agent to the patient;
- (2) obtaining a plasma sample from the patient;
- (3) incubating the plasma sample with an effective amount of ~~anti-6-keto-PGF_{1α} primary antibody~~, a secondary antibody and ~~6-keto-PGF_{1α}-aequorin conjugate~~;

an anti-6-keto-prostaglandin F_{1α} (6-keto-PGF_{1α}) antibody; an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF_{1α}-antibody; and a conjugate comprising 6-keto-PGF_{1α} covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys → Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 → Cys), 70 (Gly70 → Cys), 74(Gly74 → Cys) or (Glu76 → Cys), and

wherein the 6-keto-PGF_{1α} is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine;

- (4) removing any unbound ~~primary antibody anti-6-keto-PGF_{1α} antibody~~ and ~~6-keto-PGF_{1α}-aequorin~~ said conjugate from the plasma sample following incubation; and

- (5) measuring and correlating light intensity of the plasma sample with amount of prostacyclin within the plasma sample.

21. (Cancelled).

22. (New) A kit for measuring prostacyclin in plasma comprising:

- (1) an anti-6-keto-prostaglandin $F_{1\alpha}$ (6-keto-PGF $_{1\alpha}$) antibody;
- (2) an anti-immunoglobulin antibody that binds to the anti-6-keto-PGF $_{1\alpha}$ -antibody; and
- (3) a conjugate comprising 6-keto-PGF $_{1\alpha}$ covalently bound to an aequorin mutant;

wherein said aequorin mutant comprises serine substitutions for all three cysteine residues as present in wild-type aequorin (Cys \rightarrow Ser), wherein said aequorin mutant further comprises a single cysteine residue substituted at amino acid position 69 (Ala69 \rightarrow Cys), 70 (Gly70 \rightarrow Cys), 74 (Gly74 \rightarrow Cys) or (Glu76 \rightarrow Cys), and wherein the 6-keto-PGF $_{1\alpha}$ is coupled to the aequorin mutant via reaction with the sulfhydryl group of the single cysteine.